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Interaction of the Myelin Basic Protein with the Anionic Detergent Sodium Dodecyl Sulphate

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The interaction of the myelin basic protein and two peptides derived from it with the anionic detergent SDS (sodium dodecyl sulphate) was studied. At molar ratios of detergent/protein of up to approx. 20:1 the transient increase in turbidity (as measured by increases in A_{230}) is proportional to the ratio. Between ratios of 30:1 and 100:1 the effect of the detergent is constant and maximal. At molar ratios exceeding 100:1 the transient increase in turbidity decreases with increasing amounts of detergent. With increasing ionic strength the rapid development of turbidity is inhibited, whereas the slow decay of turbidity is not affected. Neither of the peptide fragments produced by cleavage of the myelin basic protein at the single tryptophan residue, nor both when mixed, produce measurable turbidity when mixed with SDS. Under similar conditions poly-L-lysine of similar molecular size to the basic protein shows the increase in turbidity but not the decay. The interaction between the protein and SDS is interpreted in molecular terms, which involve the initial ionic interaction of the detergent with protein resulting in aggregation and turbidity in the solution. Within the aggregated complexes molecules rearrange to maximize hydrophobic interactions.

The physical and chemical properties of the basic protein of the myelin sheath of central nervous tissue have now been investigated in considerable detail (reviewed by Carnegie & Dunkley, 1975). Although the basic protein appears to have no classical α- or β-structure (Eylar & Thompson, 1969; Palmer & Dawson, 1969; Chao & Einstein, 1970; Anthony & Moscarello, 1971; Liebes *et al.*, 1975), a specific folded conformation seems to be maintained in the molecule (Epand *et al.*, 1974; Whitaker, 1975; McNamara & Appel, 1976). The basic protein interacts specifically with acidic lipids (Palmer & Dawson, 1969; Banik & Davison, 1974; Jones & Rumsby, 1977) to produce complexes in which α-helical structure in the protein can be detected. The sites for ionic and hydrophobic interactions with lipid seem to occupy different regions of the polypeptide chain of the basic protein molecule (London & Vossenberg, 1973; London *et al.*, 1973; Jones & Rumsby, 1977).

During an investigation of the effect of the anionic detergent SDS on the fluorescence properties of the basic protein (Jones & Rumsby, 1975) we observed that at certain concentrations of detergent the basic protein was precipitated. SDS is known to induce the formation of α-helical structure in the basic protein molecule on interaction (Anthony & Moscarello, 1971). Further, the precipitation of the basic protein on interaction with SDS has been previously noted by Liebes *et al.* (1976). The present paper analyses the interactions that occur between the detergent and the myelin basic protein.

Abbreviation used: SDS, sodium dodecyl sulphate.

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Experimental

Myelin was isolated from bovine brain tissue (Rumsby *et al.*, 1970), and the basic protein recovered from the purified membrane by the procedure of Banik & Davison (1973). The basic protein prepared in this way required further purification by gel-exclusion chromatography, and this and the isolation and characterization of two peptide products from the basic protein have been described previously (Jones, 1975; Jones & Rumsby, 1977). Sodium dodecyl sulphate was of analytical grade (BDH, Poole, Dorset, U.K.) and poly-L-lysine (av. mol.wt. 23000) was from Sigma (London) Chemical Co., Kingston-on-Thames, Surrey, U.K. Both were used without further purification.

The turbidity of solutions was determined by measuring the increase in absorbance at wavelengths where light-scattering greatly exceeded the true absorption of the chromophores present. The low absorption of the chromophores used in the present study allowed the use of extremely low wavelengths, which are very sensitive to small

changes in scattering ability: 230nm was used, since this produced absorbance changes of up to 2 units. Myelin basic protein was at a concentration of 50μg/ml in Tris/Cl buffer (10mM, pH7.0). The A_{230} of this solution before the addition of the detergent was 0.1–0.2 for a 1cm-path-length cell. Possible changes in the absorbance characteristics of the chromophores (which were not determined) were thus expected to be very small in comparison with the changes produced by the increase in turbidity and were subsequently neglected. The changes in absorbance represent a sensitive and reproducible index of turbidity, although further quantification (in terms of size, shape and number of particles) was not attempted. The changes observed are critically dependent on the geometry of the sample compartment of the spectrophotometer used, more specifically on the solid angle of forward scattered light collected by the photomultiplier in the instrument. This means that measurements made on a specific spectrophotometer may be compared directly only with other results made on the same make of instrument. Two different spectrophotometers were used throughout this study: these were the Unicam SP.1800 and the Zeiss M4QIII/PMQII. Although both have long and comparable cell-to-detector-window distances, results obtained from the two are reported separately.

Experiments were initiated by adding concentrated SDS stock solutions on a plastic paddle, which was then used to mix the contents of the spectrophotometer cell at regular intervals. The temperature was 18–20°C throughout and 1 or 2ml of myelin basic protein was used in each experiment. Two SDS stock solutions (0.8 and 0.08%, w/v) were used, and blanks, without protein, produced changes of the order of 0.01 absorbance unit.

Protein–SDS aggregates were examined, as soon as maximum turbidity had been achieved, by negative-staining electron microscopy. A drop of the reaction mixture was placed on a Formvar-coated carbon-shadowed 200-mesh copper grid and left for 2–3min. Excess fluid was drawn off with filter paper. A drop of fresh 1% (w/v) potassium phosphotungstate, pH7.0, was added and left for 2min. The stain was then drawn off and the grid desiccated before examination in an AEI EM-6B electron microscope.

Results

The change in A_{230} with time after the addition of stock SDS solution to solutions of the myelin basic protein is shown in Fig. 1. Results have been corrected for blank and initial chromophore contribution and represent the turbidity of the solutions. Several observations can be made from these sample traces: 1, the precipitation of the myelin basic protein–SDS complex is transient, with a slow decay after a relatively rapid rise; visual inspection of the cuvette showed no settling out, and stirring caused no detectable rise in absorbance; 2, the time taken to reach maximum turbidity decreases as the molar ratio of detergent/protein increases, up to a ratio beyond which the time to reach maximum turbidity increases with molar ratio; 3, the maximum absorbance change initially increases as the molar ratio is increased, reaches a plateau and then decreases as the ratio is increased further; 4, results obtained at SDS/protein ratios of 18:1, 26:1 and 39:1 all display identical decay elements even though the formation of the aggregates that they represent followed different time courses.

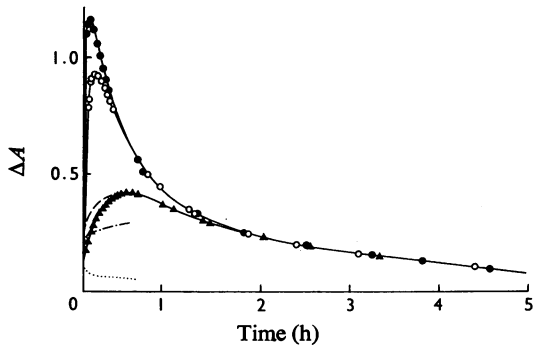

Fig. 1. *Time course of absorbance changes for myelin basic protein interaction with increasing concentrations of SDS*
Typical time-course results of A_{230} changes (Zeiss spectrophotometer) at 20°C from interaction of the myelin basic protein (50μg/ml, 10mM-Tris/Cl buffer, pH7.0) with increasing concentrations of detergent. Further practical details are described in the text. The SDS/myelin basic protein molar ratios are as follows: ▲, 18:1; ○, 26:1; ●, 39:1; ----, 130:1; -·-·, 260:1; ····, 390:1.

Data like those in Fig. 1 accumulated from many experiments with the Zeiss and Unicam spectrophotometers are plotted in Figs. 2(*a*) and 2(*b*) respectively in the form of maximum change in absorbance against molar ratio of detergent to protein. The effect of sample-compartment geometry on the absolute magnitudes of these changes precludes direct comparison, but the overall shapes of the two curves and the critical ratios agree well. Both curves (Figs. 2*a* and 2*b*) show that the plateau region where the effect is maximal and constant lies in the detergent/protein molar range of 20:1–100:1. These values include points obtained from experiments using two different concentrations of SDS as stock solutions. The critical micellar concentration of SDS is about 8mM (0.2%, w/v) at zero ionic strength (Emerson & Holtzer, 1965) and the two stock solu-

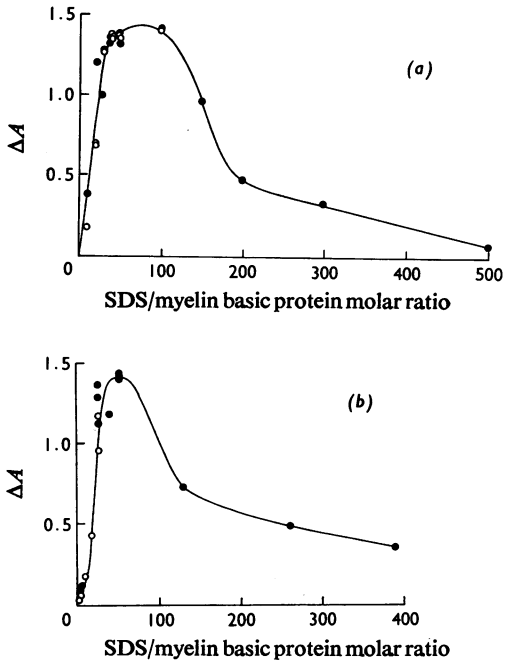

Fig. 2. *Maximum absorbance increase as a function of the molar ratio of SDS/myelin basic protein*
Conditions for interaction were as described in Fig. 1 and in the text. Changes in absorbance shown in (*a*) and (*b*) were measured on the Zeiss and Unicam spectrophotometers respectively. Two concentrations of SDS were used to obtain each result; these were (w/v) 0.8% (●) and 0.08% (○), above and below the critical micellar concentration for the detergent respectively.

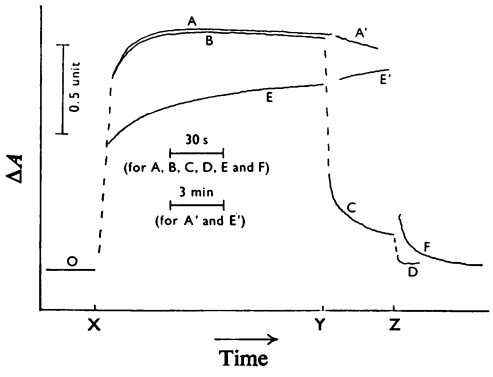

Fig. 3. *Sample traces for interaction of myelin basic protein with SDS showing the effect of excess detergent on the decay of myelin basic protein–SDS aggregates*
Conditions for interaction were as described in Fig. 1 and the text. Results in this Figure were obtained with the Unicam SP.1800 instrument at 230nm. Time-scale differences between the results in this Figure and in Fig. 1 and between curves A–F and A′ with E′ should be noted. The results were obtained with the following additions to the myelin basic protein standard solution.

Curve	Addition	At time	SDS/protein molar ratio
O	1ml of myelin basic protein in buffer	—	0
A	O+4μl of 0.8% SDS	X	41
B	Duplicate of A		41
C	As A+50μl of 0.8% SDS	Y	560
D	As C+50μl of 0.8% SDS	Z	1080
E	As O+1μl of 0.8% SDS	X	10
F	As E+30μl of 0.8% SDS	Z	330

tions used were 0.8% and 0.08% (w/v). They were thus above and below the critical micellar concentration of the detergent respectively. The results obtained by using the two stock SDS solutions lie essentially on the same curve, indicating that scattering from micelles does not contribute significantly to the changes observed. Typical traces from the Unicam recorder (Fig. 3) illustrate the effect of increasing the SDS concentration after maximum turbidity is achieved. The effect is to speed up the decay and to clear the solution rapidly.

The effect of increasing the ionic strength on the interaction of SDS and the protein is shown in Fig. 4 for two different SDS/protein molar ratios. Although at these ratios the magnitude of the changes was only reproducible to approx. 8%, the results clearly show that the interaction of detergent with the myelin basic protein is inhibited at high ionic strength. The decay rates of the aggregates formed under conditions of differing ionic strength were apparently not affected.

The two peptide fragments produced by cleavage of the basic protein at the single tryptophan residue were also examined in this system. When either fragment (at 50μg of protein/ml), or both together, were mixed with SDS at detergent/protein molar ratios of 1, 10, 100 and 1000:1 there was no measurable change in the A_{230} of the system.

Examination of SDS–myelin basic protein aggregates by negative-staining electron microscopy revealed the presence of amorphous particles ranging from some 5 to 100 nm in diameter. These particles frequently appeared to have been formed by the coalescence of several smaller aggregates.

The interaction of SDS with poly-L-lysine, a synthetic basic polypeptide of similar size to the myelin basic protein but more basic, was studied. Addition of SDS to solutions of poly-L-lysine at a concentration of 50μg/ml and with similar molar ratios to those used with the intact myelin basic protein, also caused the rapid appearance of turbidity. However, with poly-L-lysine aggregation of protein and detergent

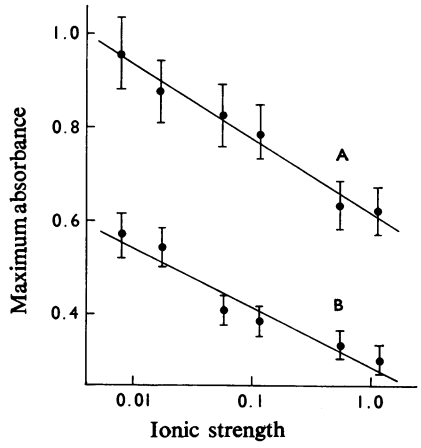

Fig. 4. *Maximum absorbance increase on interaction of SDS and myelin basic protein as a function of ionic strength*
Conditions for interaction between detergent and protein were as described in Fig. 1 and in the text except that the molar ratio for detergent to protein was 50:1 in A and 25:1 in B. The ionic strength was adjusted with NaCl. Results from two separate experiments are plotted as means ± S.E.M.

continued until the cuvette contained several particles of a few mm in diameter, which settled out rapidly. Excess SDS (up to a molar ratio of 10000:1 and beyond) did not bring about the solubilization of these complexes, which showed no signs of decay after several days.

Discussion

The interaction between the myelin basic protein and SDS is complex and depends both on time and on the detergent/protein molar ratio. The interaction is distinctly biphasic. At molar ratios of up to 20:1 the turbidity produced seems directly proportional to the amount of detergent added. Between ratios of 20:1 and 100:1 it is essentially independent of detergent concentration. Beyond this latter ratio excess SDS tends to solubilize the detergent–protein complexes. Thus, on either side of a critical range, it is possible to obtain solutions that have scattering properties that can satisfy certain (unspecified) criteria for 'tractability' (Liebes *et al.*, 1976). Anthony & Moscarello (1971) used a 150:1 SDS/myelin basic protein molar ratio for precisely this reason: precipitation occurred at lower ratios (M. A. Moscarello, personal communication).

Two major conclusions can be drawn about the interaction of the myelin basic protein with SDS: (1) ionic strength only affects the development of turbidity; (2) aggregates decay at similar rates however they have been formed. The first conclusion suggests that the initial interaction between SDS and myelin basic protein is ionic. This is confirmed by the observations of Liebes *et al.* (1976), where the molar ratio of detergent to protein required to produce a certain degree of turbidity increased as the charge on the protein increased. The similar decay patterns shown by the aggregates formed under very different conditions in this present study suggests that the same process is occurring and that it is unaffected by ionic strength.

The precipitation behaviour of other proteins with SDS shows characteristics similar to those reported here for the myelin basic protein. Thus for several globular proteins below their isoelectric points Putnam & Neurath (1945) have identified three 'zones' of interaction with detergent: (*a*) protein excess, where little or no precipitation occurred; (*b*) equivalence, a range of molar ratios where maximum precipitation occurred; (*c*) detergent excess, where the precipitate was solubilized. It was concluded that ionic interactions between protein and detergent are important in the precipitation reaction. Bigelow & Sonenberg (1962) and Imanishi *et al.* (1965) have reported similar patterns of interaction for ribonuclease and Bence-Jones protein respectively. The ionic-strength effect observed in the present work emphasizes the importance of ionic-bond formation in the initial stages of the myelin basic protein–SDS interaction. Addition of detergent will cause an increase in the concentration of Na^+ ions present in the medium. This alteration in the ionic strength could lower the critical micellar concentration of the detergent so that micellar formation contributes to the observed increase in absorbance. However, addition of detergent below (open circles) or above (solid circles) the critical micellar concentration (Fig. 2) has no effect on the shape of the curve of absorbance rise and decay, and further, the difference in absorbance between SDS solutions below (0.08%) and above (0.8%) the critical micellar concentration was only 0.01 unit. There is thus no contribution to the observed increase in absorbance from the formation of detergent micelles as Na^+ is increased.

Ionic interaction of SDS and myelin basic protein leads to protein molecules of increased hydrophobicity, which therefore aggregate producing the observed turbidity. This phase of the reaction should show significant temperature-dependence, as entropic changes must occur where cross-linking of paraffin chains takes place. The binding of large organic anions, including SDS, to albumin was explained by concluding that some detergent molecules could bind to two albumin molecules at the same time, i.e. hydrophobically to one and ionically to the other (Ray *et al.*, 1966). Such cross-linking may also account for the observed SDS–myelin basic protein aggregation.

This is the first report of the decay of a protein–SDS complex. We have previously shown (Jones & Rumsby, 1975) that at very high molar ratios of SDS to protein hydrophobic effects become important. Also the basic protein has at least one site of sufficient hydrophobicity to enhance the fluorescence of 8-anilinonaphthalene-1-sulphonic acid (Feinstein & Felsenfeld, 1975). Anthony & Moscarello (1971) have concluded from physical studies on myelin basic protein in the presence of various amphiphilic molecules that both ionic and hydrophobic interactions were important in the conformational changes observed. Liebes *et al.* (1976) have concluded that the broadening of aliphatic and aromatic resonances in the proton-magnetic-resonance spectrum of the myelin basic protein observed on interaction with SDS arises from hydrophobic effects. We therefore propose that the slow decay of myelin basic protein–SDS aggregates is due to the internal rearrangement of the molecules in the complex in such a way as to maximize hydrophobic interactions. SDS molecules cross-bridging protein molecules rearrange on to the same myelin basic protein molecule, and detergent—protein aggregates disperse slowly, probably because molecular motion within aggregates is restricted. Excess free detergent can solubilize the complexes by displacing cross-bridges. The behaviour of poly-L-lysine, which has no hydrophobic residues and which therefore cannot decay by the same mechanism, provides indirect support for the role of hydrophobic-bond formation.

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References

Anthony, J. S. & Moscarello, M. A. (1971) *Biochim. Biophys. Acta* **243**, 429–433

Banik, N. L. & Davison, A. N. (1973) *J. Neurochem.* **21**, 489–494

Banik, N. L. & Davison, A. N. (1974) *Biochem. J.* **143**, 39–45

Bigelow, C. C. & Sonenberg, M. (1962) *Biochemistry* **1**, 197–204

Carnegie, P. R. & Dunkley, P. R. (1975) *Adv. Neurochem.* **1**, 95–135

Chao, L. P. & Einstein, E. R. (1970) *J. Neurochem.* **17**, 1121–1132

Emerson, M. F. & Holtzer, A. (1965) *J. Phys. Chem.* **5**, 3718–3721

Epand, R. M., Moscarello, M. A., Zierenberg, B. & Vail, W. J. (1974) *Biochemistry* **13**, 1264–1267

Eylar, E. H. & Thompson, M. (1969) *Arch. Biochem. Biophys.* **129**, 468–479

Feinstein, M. B. & Felsenfeld, H. (1975) *Biochemistry* **14**, 3049–3056

Imanishi, A., Momotaji, Y. & Isemura, T. (1965) *J. Biochem. (Tokyo)* **57**, 417–429

Jones, A. J. S. (1975) D.Phil. Thesis, University of York

Jones, A. J. S. & Rumsby, M. G. (1975) *J. Neurochem.* **25**, 565–572

Jones, A. J. S. & Rumsby, M. G. (1977) *Biochem. J.* **167**, 583–591

Liebes, L. F., Zand, R. & Phillips, W. D. (1975) *Biochim. Biophys. Acta* **405**, 27–39

Liebes, L. F., Zand, R. & Phillips, W. D. (1976) *Biochim. Biophys. Acta* **427**, 392–409

London, Y. & Vossenberg, F. G. A. (1973) *Biochim. Biophys. Acta* **307**, 478–490

London, Y., Demel, R. A., Geurts van Kessel, W. S. M., Vossenberg, F. G. A. & van Deenen, L. L. M. (1973) *Biochim. Biophys. Acta* **311**, 520–530

McNamara, J. O. & Appel, S. H. (1976) *Neurology* **26**, 387

Palmer, F. B. & Dawson, R. M. C. (1969) *Biochem. J.* **111**, 637–646

Putnam, F. W. & Neurath, H. (1945) *J. Am. Chem. Soc.* **66**, 692–697

Ray, A., Reynolds, J. A., Polet, H. & Steinhardt, J. (1966) *Biochemistry* **5**, 2066–2075

Rumsby, M. G., Riekkinen, P. J. & Arstila, A. V. (1970) *Brain Res.* **24**, 495–516

Whitaker, J. N. (1975) *J. Biol. Chem.* **250**, 9106–9111